The Importance of the Measurement of Enzyme Activity in Botanical and Agricultural Chemistry

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For many branches of botanical and agricultural chemistry it is important to be able to determine enzyme activity in soil and plants. The amount and activity of the enzymes depends on the nature of the soil, on the fertilizer used and on the method of cultivation during the vegetative period[1]. The activity of the enzymes is of importance for the quality, stability on storage, germinating power and other properties of plants. The activity of the enzymes is determined and not the quantity[2,3].

Plants root in soil and obtain their requirement of water and mineral salts from it. It is not a dead substrate according to the definition of *Liebig*. Soil without micro-organisms, and therefore without enzymes, does not usually provide a satisfactory growth medium for higher plants. The classical microbiological methods for the estimation of bacteria (bacterial count according to *R. Koch*) and their activity fail to work when applied to soil. The amount, nature and activity of the enzymes in soil (which are found there because of the presence of micro-organisms) is of greater interest for the evaluation of soil than bacterial counts. In addition, enzyme assays are simpler to carry out and give exact and comparable values.

I. Enzyme Activity in Soil

The root system and aerial parts of plants become incorporated into the soil on the death of the plant. The type and extent of their bacterial decomposition determines the quantity and the quality of the humus formed, which is of importance for the fertility of the soil. The enzymes contained in soil can be divided into ectoenzymes and endoenzymes.

Ectoenzymes are released from living micro-organisms. They attack organic substances of high molecular weight, which cannot be absorbed by the bacteria.

Endoenzymes occur in the soil after the death of the micro-organisms. Because soils usually have a high content of clay and humic substances, they have a high buffer and absorption capacity. Dead micro-organisms do not decay by putrefaction, but rather by autolysis which liberates the endoenzymes. They withstand decomposition (adsorbed on clayey minerals) and remain active for a long period.

Consequently a profile of enzyme activity occurs in soil, which is produced by the micro-organisms and is maintained by them. The profile is to a slight extent contributed to by the enzyme content of the bacteria, which are living in the soil at the time of the collection of the sample. This contribution is subject to the same seasonal and other variations as the bacterial count. By far the largest fraction of the enzymes are excreted ectoenzymes and endoenzymes which are set free by the autolysis of the older, dead bacteria and are retained in the soil.

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[1] *E. Hofmann, A. Seegerer, E. Latzko, K. Bräunlich* et al., Biochem. Z. *321*, 97 [1950]; *322*, 174 [1951]; *324*, 125, 308, 397 [1953]; *325*, 329 [1954].

[2] *E. Hofmann*, Landwirtsch. Forsch. 7. Sonderheft, p. 80 [1955].

[3] *G. Hoffmann*, Z. Pflanzenernähr., Düng., Bodenkunde *85*, 97, 193 [1959].

Therefore the level of enzyme activity in soil is not subjected to the same variations as bacterial counts. It changes only slowly, and then only when the number of micro-organisms is increased or lowered for a long period due to the method of cultivation. Natural phenomena make no difference or are scarcely noticeable.

For the evaluation of the measured enzyme activity it is necessary to take into account the type of soil (sand, loam or humus) and the type of cultivation in recent years. Sandy soil has only slight, loamy soil average and humus high enzyme activity. In addition, the pH of the soil has a great effect. Acid soils contain relatively few micro-organisms and consequently only low enzyme activity. The nearer the pH is to neutrality, the higher is the enzyme activity. In arable and garden soils the enzymes are evenly distributed in the topmost layer of the soil (about 20 cm.) because of frequent cultivation. Their activity is only half as much as in pasture soil of a similar type, where the enzyme activity is restricted to the upper, well rooted layer of about 5 cm. and rapidly falls off with increase in depth ("enzyme profile of the soil"). Organic substances (remains of roots, stable manure, green manure, peat and humus substances) increase the enzyme content by promoting the growth of the bacteria. The same is true of efficient tilling of the soil (aeration). Bad aeration and excessive cultivation are injurious, the former by reducing and the latter by temporarily increasing oxidative processes which destroy organic substances and therefore very soon stop the growth of the micro-organisms.

Special cases are garden soils, forest soils and soils which have been specially cultivated for many years (soil on which hops have been grown). About twice the enzyme activity is found in garden soils as in arable soils. After many years of one crop culture (*e.g.* hops, asparagus, strawberries) the enzyme activity falls to about half that in adjacent arable soils because of the good manuring and cultivation. Only the urease activity increases (up to double), due to the use of large amounts of stable manure and other organic substances.

II. Enzyme Activity in Plants

The determination of enzyme activity in plants provides information about the effects that fertilizers, control of pests, storage at different temperatures, germination and other conditions have on the plant. From the activities of different enzymes in plants, growing and after harvest, it is possible, for example, to discover a suitable fertilizer. An increased activity of the oxidative enzymes[4] is detrimental to the quality and quantity of the harvest. Increased respiration of plants during storage is linked to large losses of matter. The amount of oxidases closely parallels the activity of the hydrolytic enzymes.

III. The Individual Enzymes

The following enzymes are most frequently measured in soil and plants.

Invertase

Invertase[1] (see p. 904) should be determined initially in all cases, because the method is simple, gives very accurate values and is very informative about the biochemical processes in the soil.

Soil: The amount of invertase closely parallels the bacterial content of the soil and their metabolic activity. Different types of soil have the following invertase activity:

[4] *A. Amberger*, Landwirtsch. Forsch., 6. Sonderheft, p. 134 [1955]; 14. Sonderheft, p. 107 [1960].

Sandy soil of little value; damp, cold and acid loamy soil;	
damp, acid, marshy soil	under 7 units
Good, neutral or slightly acid, arable soils (sandy and mild loams)	7—15 units
Very good arable soils in the top soil to *ca.* 20 cm. deep; all good pasture soils in the upper 5 cm.	over 15 units
Acid, uncultivated, high moor soils	very slight to slight activity
Neutral, lime-containing, marshy soils (low moor soils)	very high activity

Plants: In the growing plant the hydrolytic enzymes are necessary for the metabolism of the initial assimilation products. Too high activity of these enzymes leads to loss of carbohydrate and therefore decreased yields. Although seeds contain plenty of sucrose, invertase is scarcely present. However, a few days after germination the activity increases rapidly and reaches its highest value during the period of greatest growth of the plant. Then the invertase activity decreases in the vegetative part of the plant. In the reproductive part of the plant it increases further and after an optimum falls, until at maturity it is practically zero. If an unbalanced nitrogen fertilizer is used, the increase in activity during the vegetative period is above normal. The plants are then deficient in carbohydrate. The invertase activity reaches its normal level if a balanced fertilizer containing potassium and other plant nutrients is used. The invertase activity is therefore a very sensitive indicator of normal growth and the correct use of fertilizers.

β-Glucosidase[5] and amylase

β-Glucosides are present in large amounts in plants, especially in stalks and roots. The activity of β-glucosidase varies considerably during the vegetative period. For the assay, see p. 867. The determination of amylase activity in seeds, grains and tubers before and during germination, and in plant material during the phases of growth, is of special interest. The variations are considerably smaller than those with invertase. For the assay of amylase activity, see p. 854. The pH optimum for the enzyme from the green parts of plants is 6.0, while for the enzyme from grain it is 4.7.

For the evaluation of the activities, see the section "Invertase". The β-glucosidase values are normally about half, and the amylase values about a quarter of the invertase activity.

Urease[4,6]

Urease is present in large amounts in biologically active soil, since many micro-organisms hydrolyse urea enzymatically. Assays of urease activity (see p. 913) give information on the rate of degradation of nitrogen-containing compounds, especially proteins.

Evaluation: according to[6] the following values are obtained: 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 sample is obtained[1]. Soil with vegetation contains more urease than that without[1]: the same soil without vegetation: 7.9 units/10 g.; with grass: 16.8 units/10 g.

[5] *E. Hofmann* and *G. Hoffmann*, Biochem. Z. *324*, 397 [1953].
[6] *E. Hofmann* and *W. Schmidt*, Biochem. Z. *324*, 125 [1953].

IV. Techniques

Soil

Several small samples of soil are obtained from the area to be examined in Autumn or Spring, preferably in dry weather. Garden and arable soils are sampled to a depth of 20 cm., while in the case of pasture soils only to a depth of 5 cm. The samples are combined and well crumbled together to obtain a homogeneous sample. If it is suspected that there may be large variations in the areas of soil, the individual samples should be examined.

The samples are dried (not in sunlight) for 24 to 28 hours at 25 to 35°C and are stored in tightly stoppered powder bottles in a dry room. They keep for several months.

Two assays are usually sufficient for the determination of enzymes, providing that they give results which agree and are reproducible. The addition of toluene prevents interference from micro-organisms during the determination.

Plants [7-9]

To obtain a good average sample, large amounts of whole plants, similar organs (of similar age), seeds or fruits are collected in dry weather in the early morning, when all the assimilation products of the previous day have been transported from the leaves and before renewed assimilation occurs.

For absolute measurements the plant material must be analysed immediately after collection and without loss. Leaves, stalks, and fruits are ground, grain is milled, and tubers and roots are homogenized. The disintegrated material is analysed directly.

For comparative measurements or in the case of large numbers of samples which cannot be analysed sufficiently quickly, it is necessary to prepare the samples so that they can be stored for a long period without loss of activity. Alcohol-ether dried preparations and glycerol extracts (see p. 905, 906) are suitable for this purpose. Dry preparations are stable for several months in tightly stoppered powder bottles, while glycerol extracts are stable for several days in a refrigerator under toluene.

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7) *E. Hofmann* and *E. Latzko*, Z. Pflanzenernähr., Düng., Bodenkunde *66*, 65 [1954]; Biochem. Z. 321, 94 [1951]; Landwirtsch. Forsch., 2. Sonderheft, p. 68 [1952].
8) *A. Amberger*, Biochem. Z. *323*, 437 [1953].
9) *H. Scheck*, Z. Pflanzenernähr., Düng., Bodenkunde *60*, 209 [1953].