A NEW APPROACH FOR QUANTIFICATION OF SOIL AMIDASIC POTENTIAL

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ABSTRACT

The principle of pedo-enzymatic methods, applied in all soil researches, was published by Hofmann and Seegerer (1951) for soil saccharasic analysis and by Kuprevich (1951) for soil ureasic analysis. This principle was inspired from those applied in biochemistry of plants or animals: a mixture is prepared from a plant or animal tissue extract, the specific substratum for the researched enzyme and an antiseptic. After a certain time at a constant temperature, the product resulted from the enzymatic reaction is determined. In pedo-enzymology, in the same enzymatic process, instead of plant or animal tissue extract, a few grams of soil are introduced in the enzymatic mixture, The authors of this paper are proposing a new principle for determining the amidasic potential of soils: the enzymatic mixture does not contain the specific substratum because both the substratum (amides) and the amidases (urease, asparaginase, glutaminase and arginase) are usually present in soil, but in different ratios. These ratios determine the soil potential for ammonium release in soil. The laboratory amidasic analysis determines the quantity of ammonium released in soil in 24 hours at 28°C.

Key words: amidase, enzymatic method, pedoenzymology, soil enzymes.

INTRODUCTION

Methods for studying the hydrolasic enzyme activity in soils were inspired from those used in biochemistry of living organisms and their derivatives. Detection of enzymes in soil are at the origin of discovery of the pedoenzymatic processes. Thus, Rogers (1941) discovered that β-glycerophosphate is hydrolyzed in soil by a phosphomonoesterase, liberating phosphorus ions. This phosphatasic process was notified and explained since 1939 by Ionescu and, respectively, by Pavlovschi and Ionescu (1940). Then, the pedoenzymology age was inaugurated by Hofmann and Seegerer for beta-h-fructosidasic (saccharasic) process and also by Kuprevich (1951) for ureasic process. In the following years, the number of papers concerning pedo-enzymatic research, increased evidently, every year, according to the review of Kiss and Boaru (1965). The methods of the pedo-enzymatic analyses are based on the same principle as in biochemistry, but instead of using various animal, plant or human extracts, the soil itself is used in the following mode: in a bottle, that can be hermetically plugged, one introduces a few grams of soil (that one supposes to contain the enzyme sought), then one adds an antiseptic solution (for inhibiting soil microflora) in which the enzymatic substratum was dissolved. This bottle, hermetically plugged, is introduced in a thermostat at 28°C. After a certain time, the bottle is uncorked and a 0.3% aqueous solution of alum is added (for a better filtration) and one stirs 15 minutes and then filters. The enzymatic product is quantified in an aliquot of filtrate. In this way, the soil enzymatic potential is tested. It is proved that released enzymes (actively or inactively) are adsorbed by soil colloids and are largely protected from biodegradation.

MATERIAL AND METHODS

It is clear that these methods give information about the quantity of enzyme adsorbed on the soil colloids (regardless of its quality). Therefore, these methods only show the enzymatic hydrolytic potential, because the analyst introduces as much enzymatic substratum to satisfy (in optimum) the enzyme / substratum system, in conformity with Michaelis' constant.

Ştefanic et al. (1965) and Ştefanic (1971) proposed a method, based on a new principle, for researching the enzymatic hydrolysis of all organic compounds of phosphorus, including even meta- and pyrophosphates, which can preexist in soil, in every moment of the analysis, without adding of enzymatic specific substratum. Phosphorolysis was the results of all specific substrata preexistent in soil. This new principle was remarked by Speir and Ross (1978) who wrote: *„ …. However, hydrolysis of soil organic phosphorus by native soil enzymes was demonstrated by Stefanic (1971), who incubated toluene-treated soil without added substrate for 24 h at 28°C and measured the increase in extractable inorganic phosphate”.*

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In the present paper we are proposing to extend the above mentioned principle, for estimating total amidasic potentials of soils, instead of separate estimation of each: ureasic, asparaginasic and glutaminasic potentials, or amylasic, cellulasic, saccharasic etc. potentials, that were estimated by the old methods. All amidases hydrolyze the C – N link of amides as follows: urease hydrolyzes urea releasing NH_3 and CO_2; asparaginase releases NH_3 and aspartic acid from asparagine; glutaminase hydrolyzes glutamine with formation of NH_3 and glutamic acid and from arginine, arginase releases NH_3 and ornitine. All these enzymes were identified in the soil.

The method proposed by us, offer new opportunities, because in the soil, one can find in the same place and time, both amidases and their specific substratum (amides), even if they are not in an optimal rapport of mass. The soil water, circulating in all directions, creates the conditions to put in contact the enzymes with their substrata, even in the case when humidity deficiency limits ammonification activity of soil microflora.

Our method is more ecological, because it reflects the total amidasic potential of the soil in action.

Description of this method: 10 g from the soil sample (passed through a sieve of 2 mm and without vegetal remains) are introduced in a bottle that can be hermetically closed. Then, 10 ml of an antiseptic solution (sodium azide 0.015% in water) is added; the bottle is closed and introduced in a thermostat for 24 hours, at 28°C. This represents the active soil sample of the analysis. In the same time, an inactive soil sample is obtained, in the same mode: 10 g of soil sample is treated with 10 ml of antiseptic solution, in another bottle. Both the inactive and active samples are diluted with 15 ml of 0.3% alum in water. The two variants are stirred 15 minutes and filtered. Then, 5 ml of each filtrate are shifted in a test tube of 25 ml capacity, treated with 2 ml of Seignette salt 20% in water, one adds approximately 5-7 ml distilled water and 1 ml of Nessler reagent. The liquid is homogenized and with distilled water one adds to the volume of 20 ml. A new homogenization is done and after 20 minutes one determines, spectrophometrically, at 425 nm wave length, the content of NH_4^+ in 5 ml of filtrates of inactive and of active enzymatic mixture. Finally, one makes the difference between active – inactive enzymatic mixtures and one refers to 100 g of soil, dry matter.

Our method was verified with 3 samples from the following soil types: cambic chernozem from the park of National Agricultural Research & Development Institute Fundulea, Călăraşi; reddish preluvosoil from the park of University of Agronomical Sciences and Veterinary Medicine Bucharest, and albic luvosoil, not influenced by agro-technological factors, from Agricultural Research & Development Station Albota-Piteşti, Argeş County.

RESULTS AND DISCUSSION

In table 1 we present the quantity of ammonium released in soil by the amidasic process, during 24 hours of enzymatic reaction, at 28°C.

In the conditions of our method, the real quantity of released ammonium in soil was possible to be quantified, for 24 hours of total amidasic reaction, because in this time, the nitrification process was stopped (by the antiseptic solution) and in this way, NH_4^+ was accumulated in the enzymatic reaction bottle.

Table 1. The total amidasic potential in different soil types

Soil type	Total amidasic potential (NH_4^+, mg/100 soil d.w.)	Total N-NH_4^+ kg/ha (amidasic released)
Cambic chernozem	0.470	10,98
Reddish preluvosoil	0.650	15.18
Albic luvosoil	0.746	17.40

The standard methods for ureasic reaction, using urea as substratum (for example), produce a very large quantity of ammonium, without of any correspondence with the reality of the processes in soil. On the contrary, by our method, it is possible to approximate what is the potential of the soil (in the same conditions as in laboratory) to release ammonium by total

amidasic process. For example, in the table 1 we calculated that in chernozem, 10.98 kg of $N\text{-}NH_4^+$/hectar were released in 24 hours, in preluvosoil, 15.18 kg and in albic luvosoil, 17.40 kg, without any technological intervention. For comparison, at a dose of 180 kg/ha of ammonium nitrate, 90 kg $N\text{-}NH_4^+$/ha is delivered. This method opens the possibility to detect the influence of fertilization on the biological processes in soil.

CONCLUSIONS

The standard methods in pedoenzymology give some useful information for quantifying the biotic and enzymatic potential of soils, but this information is too restricted because it reflects more the quantity of one specific enzyme and it can not reflect the quantity of substratum preexistent in soil.

For studying the total phosphatasic potential of soils Ştefanic et al. (1965, 1971) and Irimescu and Ştefanic (1998) proposed a conceptually new, more ecological method.

The method presented in this paper, for determining the total amidasic potential of soils is also more ecological than the ureasic or asparaginasic methods recommended so far by literature.

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