Changes of enzymatic activity in soil supplemented with microbiological preparation UGmax®

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Abstract
The influence of the microbiological fertilizer UGmax® on soil biological activity measured as dehydrogenase and cellulases activities was determined. The research was carried in 2005-2008 on a productive field of 2 ha under winter wheat and winter rape. One half of the field was supplemented with UGmax® every year after harvest on the stubble and as top-dressing in spring, while the other part was the control. Ten soil samples localized with the GPS were collected every year (2005 – 2008) from the humus horizon. Analyses of basic soil chemical parameters and enzymatic activities were done prior the experiment (in 2005). The results showed that the microbiological preparation UGmax® significantly promoted the initial phase of the decomposition of post-harvest residues what was confirmed by a clear decrease of cellulase activity in the soil samples taken from the field with UGmax® as compared with control field. Cellulases activity decreased gradually year after year. The most significant drop of this activity was noted in the specimen taken in the last year. The application of UGmax® has not influenced the activity of dehydrogenases. The tendency observed over the experiment was similar in both sets of soil samples. Furthermore UGmax application increased soluble carbon content significantly which was confirmed by higher organic carbon and total nitrogen contents.

Key words
Biological preparation, cellulases, dehydrogenases, enzymatic activity, soil, UGmax®

Introduction

Organic matter is the most important soil component, always taken into account in all issues of environmental protection. Soil organic matter quality and quantity is of importance for many soil features, such as soil water retention, sorption and buffering potential, etc. Moreover, it is the source of nutrients and energy for living organisms. Since soil organic matter is one of the elements of the global matter and energy circulation, being both the producer and the emitter of CO_2, and finally its natural sequestration complex system. That is why decreasing quality and quantity of soil organic matter is extremely important, not only because of soil fertility, but also from the point of view of climate protection.

One of the methods for increasing organic matter content in arable soils is application of microbiological preparations. They increase soil microbial activity and in consequence humus compound formation. UG max produced by the "Bogdan" Trade-Service Co, Ltd. is one of microbiological preparations available in Poland. It is composed of lactic acid, *Pseudomonas* and *Penicillium* bacteria plus actinomycetes. Preliminary results showed that UG max can increase the decomposition rate of post-harvest residues. It was confirmed by a parallel decrease of cellulase activity. Moreover, UGmax increased and stabilized organic matter content and available phosphorus content.

Soil enzymes play an important role in the catalysis of some important reactions essential for soil microorganisms, decomposition and formation of organic matter, and are responsible for nutrient cycling and decomposition of organic wastes [Dick and Tabatabai 1993], what of course is of special agricultural significance. Total soil enzymatic activity is composed of both intracellular and extracellular enzymes. The dehydrogenase group of enzymes is the best example of exclusively intracellular enzymes. Dehydrogenases play a significant role in the biological oxidation of soil organic matter by transferring the proton from substrates to acceptors (Rossel *et al*. 1997). That is why their activity is considered an indicator of the oxidative metabolism in soil and thus also of microbial activity [Quilchano and Maraňón 2002]. However, the largest part of soil enzymes is extracellular and excreted to the soil solution. They are extremely important in the hydrolysis of substrates that are too large or insoluble to be taken up directly by cells (Dick

1997). One of them is cellulose, the most abundant organic compound in the biosphere, comprising almost half of the biomass synthesized by photosynthetic fixation of CO_2 (Eriksson *et al*. 1990). The hydrolytic enzymes that mediate cellulose degradation in soil are known as cellulases. There are three kinds of cellulase which can hydrolyse cellulose in different ways. Due to importance of the cellulase complex in the global recycling of cellulose it is important to understand the factors that affect the enzyme, so that it may be used more often as an index of the soil fertility status.

Material and Methods

The soils were collected from the experimental field in 2005-2008.The research concentrated on the surface (humic) horizon of eutric, gleic Cambisols of a productive field of 2 ha under winter wheat (2005, 2006, 2008) and winter rape (2007) localized in the southern part of the Sepopolska Plain near the Budniki village (54° 11' 54" N and 20° 38' 12" E). One half of the field was supplemented with UGmax® every year after harvest on the stubble (0.7 l per ha) and as top-dressing in spring (0.3 l per ha), while the other part was the control. Ten soil samples localized with GPS were taken every year (2005 – 2008) from the soil humus horizon. Results of the analyses of basic soil chemical parameters were done prior the experiment (2005) and after UGmax® application (2006-2008) (Table 1). The data on dehydrogenases and cellulases avtivities determined in 2005 were published earlier (Smolinski *et al*. 2008). Since our idea was to reach the soil equilibrium and to learn on a long-term soil quality status we analysed the soil not earlier than after six months of the UGmax application.

Soil dehydrogenases activity was measured as described by Casida *et al*. [8] with some small modifications. In general, soil samples of 6g were placed in 16mm x 150 mm test tubes and incubated with 1ml 3% 2,3,5-triphenyltetrazolium chloride (TTC), 60mg $CaCO_3$ and 2.5ml distilled water at 37°C for 24h. After incubation the red 2,3,5-triphenyltetrazolium formazan (TPF) was extracted with 50 ml of ethanol and read colorimetrically at 485nm for quantification. Cellulolytic activity was measured according to Deng and Tabatabai [1994] with some modifications as given herewith. 5 g of air-dried soil samples were weighed and rinsed with 1 ml toluene in order to stop microbial growth. Than the samples were suspended in 5ml 10% carboxymethyl cellulose (CMC - sodium salt) in 50 mM acetate buffer of pH 5.5 and incubated at 30°C for 24 hours. After that the amount of reducing sugars released was determined with the standard Nelson-Somogyi colorimetric method as described by Deng and Tabatabai [1994]. All determinations were made in triplicate and expressed on a dry weight basis (DM). Results of dehydrogenase and cellulolytic activities were expressed as □M TPF/g DM ·/24h and □M glucose ·/g DM ·/h x 10^{-3}, respectively.

Results

As is shown in Table 1, UGmax® application caused an increase of soil organic matter content and also stabilized it, what was confirmed as increased organic carbon and total nitrogen concentrations. Preliminary results showed that the microbiological preparation (UGmax®) under study clearly accelerated the initial phase of post-harvest residues decomposition. It was confirmed by a distinct decrease of cellulose activity in the soil samples taken from the field where the UGmax® was applied as compared with the control field. Cellulases are known to be the inductive enzymes and their activity depend on the substrate availability and products content. UGmax accelerated the fresh organic material (straw) decomposition and simultaneously declined the amount of the substrate available for cellulose degradation. As the result a significant enzymatic activity decreasing was noted after second (spring) UGmax® application. A significant decrease of cellulolytic activity was observed in the soil samples collected after one year of the UGmax® application with the mean activity of 0.737μM glucose /1g d.m. soil/1h 10^{-3}. Enzymatic activity decreased systematically year after year. The most significant decrease of its activity was noted in the soil taken in the last year of the experiment (Figure 1).

Table1. Some chemical properties of soil under study (2005-2008)

Parameter	2005				2006-2008			
	with UGmax®		without UGmax®		with UGmax®		without UGmax®	
	mean	min-max	mean	min-max	mean	min-max	mean	min-max
C_{ORG} [g/kg]	15.44	14.15-16.46	15.42	12.30-19.51	17.3	13.28-27.16	14.76	12.01-19.40
N_{TOT} [g/kg]	1.52	1.41-1.64	1.51	1.23-1.85	1.70	1.32-2.51	1.44	1.41-1.64
Clay fraction [%]	24.0	15.0-29.0	22.4	16.0-31.0	24.0	15.0-29.0	22.4	16.0-31.0
pH 1M KCl	6.16*	5.82-6.51	5.94*	5.51-6.62	6.19*	5.51-6.84	5,35*	4.46-5.96

*geometric mean

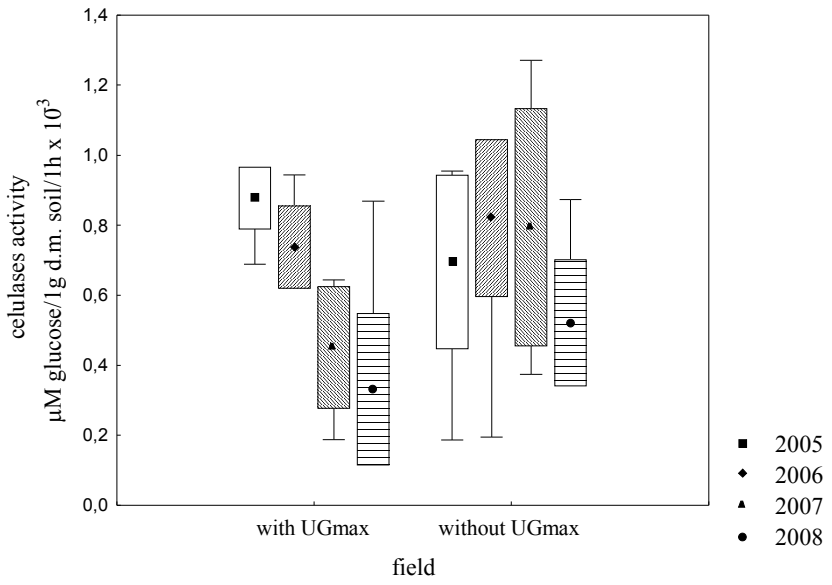

Figure 1. Changes of cellulases activity in the field with and without UGmax®

The mean value of cellulases activity in the soil samples taken from the field with UGmax® in 2008 amounted 0.331µM glucose /1g d.m. soil/1h 10^{-3}. The total reduction of studied enzymes activity after UGmax® using reached 0.55µM glucose /1g d.m. soil/1h 10^{-3} (Figure 2), while cellulases activity determined in the soil samples taken from the control plot changed negligibly. The only significant change in their activity was observed in samples analyzed in the last year of the experiment. In this case the activity decreased about (of) 0.17 µM glucose /1g d.m. soil/1h 10^{-3} (Figure 1).

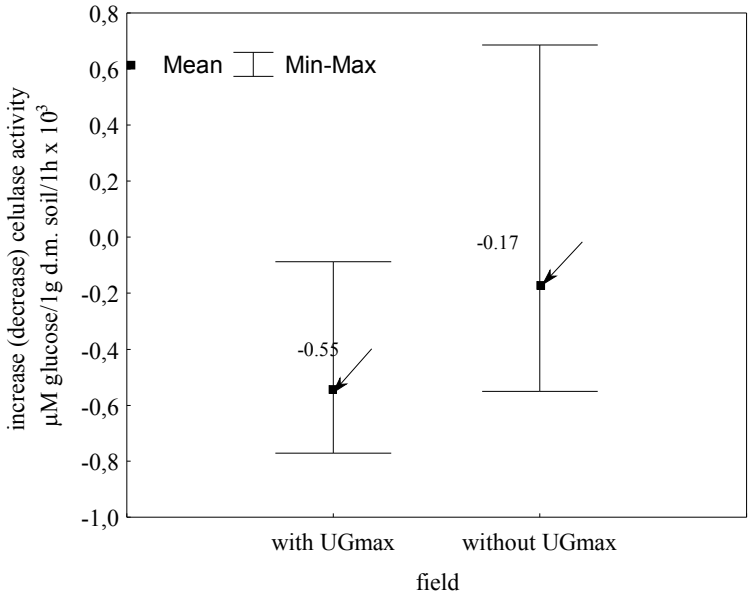

Figure 2. Cellulases activity before and after the UGmax® application (2005-2008)

Results of cellulolytic activity exhibited a high spatial variability within the analyzed area. It was confirmed by a significant dispersion of results among soil samples. The reason for that phenomenon could be probably differentiation of chemical parameters in the surface soil horizon (Table1).
The application of the UGmax® did not affect the activity of dehydroegenases activity very little. The differences occurring in soil samples taken every year were similar in both fields. The highest dehydrogenases activity was noted in the soil samples taken in 2007, both in samples with UGmax® and without it. Dehydrogenases activity, similarly to that of cellulases, disclosed a significant spatial variability.

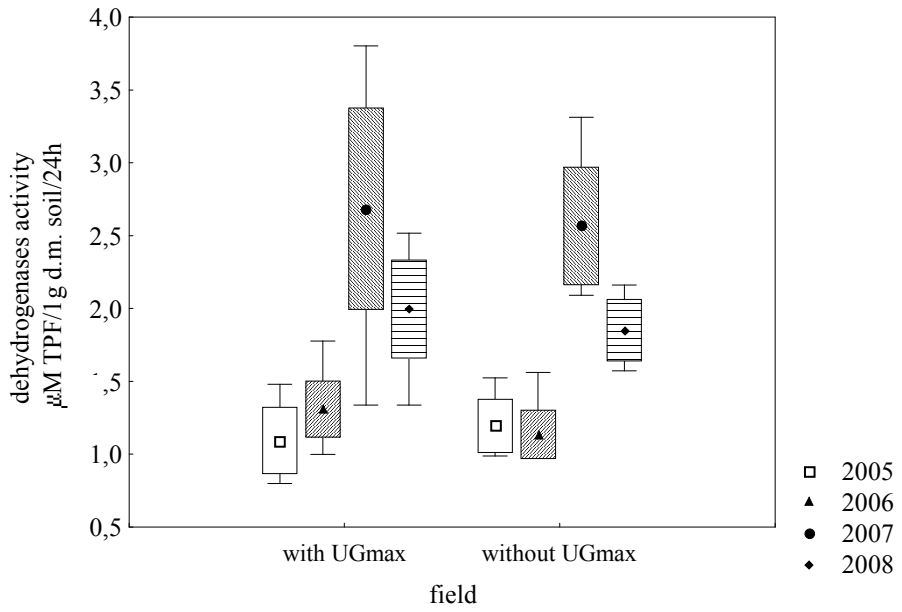

Figure 3. Changes of dehydrogenases activity in the field with and without UGmax

Conclusions

The results showed that UGmax® is the preparation determining the decomposition rate of post-harvest residues and increasing significantly the amount of soil organic matter. It can be concluded that out of the enzymes studied only the activity of cellulases can be a good indicator of soil changes after the UGmax® use because these enzymes clearly and univocally respond to the preparation application. The application of the UGmax® had basically no influence on the activity of dehydrogenases. There was no clear tendency in the dehydrogenases activity in soil samples taken from both UGmax® field and the control one.

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