

## Synthesis and Decomposition Processes of Humic Compounds in Soil

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Humic compounds have not only fundamental significance in the formation of soil structure, but they also take part in the regulation of plant mineral nutrition.

As experiments have shown, mineral nitrogen fertilizers are more effective in soils rich in humus. Such soils have a large microbial population, which assimilates part of the nitrogen fertilizers diminishing this way losses by leaching and denitrification. Here we have biological fixation and reservation of nitrogen for a short time. When the bacteria die nitrogen is transformed into the mineral form, accessible for plants.

In our report we are not setting ourselves the task to analyse the biochemical process of humus formation, only to elucidate the question about the place of this process in the soil, which has a bearing on agricultural production.

The fact that nutritive minerals are more readily available for the plants in the upper horizon can be shown by experiment. In the soil samples

*Table 1*

The germinating capacity of wheat grains in different layers of chernozem soil

The method of soil tillage in the last 5 years		The layers of soil /centimeters/				
		0 - 5	5-10	10-20	20-30	30-40
Turning over /in the depth of 25 cm/	% germinated grains	80	53	45	25	10
	The height of seedlings	119	73	65	45	10
Without turning over /in the depth of 8 cm/	% germinated grains	90	66	50	53	40
	The height of seedlings	67	50	50	41	30

taken from the upper horizon plants grow appreciably better, than in the ones drawn from deeper horizons.

In Table 1 we set out the results of an experiment with chernozem soil, the humic horizon of which was 40 centimetres thick. The samples taken from the different layers of this very fertile soil, which was moistened to 70%, were put into Petri dishes. 30 grains of wheat were sown in every dish. The quantities of germinated grains and the height of the seedlings were determined after 3 weeks. The results show that the best conditions for plant growth were ensured in the upper horizon of the soil /VOSTROV, 1960/.

Summing up the above data, it is possible to conclude that in the upper horizon of cultivated soils under the conditions of microbial activity humus decomposition takes place. With the purpose of restoring its fertility it is expedient to turn the soil over. In this way it is possible to enrich the exhausted upper soil layer. But the experiments conducted in our laboratory have also shown that the place where more active humification is going on is the upper horizon of the soil. In the course of the experiments ground wheat straw was added to sand and this mixture was put into a tall vessel. After 6 months the different layers of sand were analysed in the presence of humous substances. It was established that the most active formation of humus took place in the upper horizon, more rich in microbial population.

Thus the question of how to enrich a cultivated layer with humic substances is answered by working organic materials into the soil. The method of tillage approach to soil treatment however must be chosen to suit local conditions.

Discussing humus decomposition, we shall dwell on the change of the cenosis of microorganisms during the decomposition of plant remnants and after that we will turn to the destruction of humic compounds.

Summing up the results of model experiments, conducted until the present time, it can be noted, that in the first phases of the decomposition of plant remnants a mass growth of nonsporiferous bacteria, dominated by the group of *Pseudomonas*, has been observed. Later on spore-forming bacteria and actinomycetes carry on jointly their destructive activities. Among the latter we pay special attention to *Nocardia*-like actinomycetes which are usually neglected during analyses because they produce atypical colonies on rich nutrient media. They can be observed only on poor media, as for example, on nitrite-agar or on agarized soil extract. On such media they form colonies with mycelium and are easily revealed. At first, *nocardia* appear in great quantities as yellow pigmented colonies /group

Table 2

The succession of groups of microorganisms in the course of the decomposition of plant remnants /model experiment with haricot residues/

Microorganisms	The quantity of cells of microorganisms /10 <sup>5</sup> /lg/ after days of decomposition					
	1	10	30	45	60	270
Total amount of saprophytes	22	2760	1840	2300	740	270
Fluorescent forms	7	2300	1440	1300	340	200
Spore-forming bacteria	5	440	400	1040	400	70
<i>Nocardia</i> :	-	28	-	45	-	28
<i>Øerscovia</i>	-	28	8	9	-	-
<i>Rhodococcus</i>	-	-	2.5	4	-	28

Rhodococcus/. The changes in the quantity and composition of microorganisms in the course of destroying the plant residues are shown in Table 2.

The study of the decomposition of fulvic and humic acid specimens in liquid mineral medium and gel medium, saturated with given compounds, has brought to light the presence of Rhodococcus cells among the growing microorganisms. These observations led to the conclusion, that microorganisms of the genus Rhodococcus are able to decompose complex organic compounds, inaccessible to other microorganisms.

Observers of *Øerscovia* and Rhodococcus cultures have found that microorganisms of the genus Rhodococcus, unlike *Øerscovia*, assimilate not only simple organic substances, but also aromatic compounds, including humic substances, causing their decoloration.

The introduction of an easily metabolized substance /for example, glucose/ into the nutrient media accelerated the decomposition of humic substances.

The physico-biochemical properties of *Øerscovia* and Rhodococcus cultures differ greatly, which makes them easier to recognize. The differences in the chemical composition of the hydrolysates of *Øerscovia* and Rhodococcus cells are also considerable /Table 3/.

It should also be noted, that on nutrient media, where aromatic and humic compounds are the only carbon source, bacteria of the genus *Pseudomonas* /*P. putida* and *P. geniculata*/ have been revealed.

Thus, the data we obtained give reason to believe that the names Rhodococcus and Pseudomonas should be added to the list enumerating the main destructors of the nuclei of humic compounds. Evidently this list may be continued, but it should be taken into account that it is necessary to distinguish between the groups of microorganisms, tearing away peripheral fragments of the molecules of humic substances, and the microorganisms, destroying the aromatic nucleus of such molecules.

Most experiments on humus destroying microorganisms were conducted with humic substances, isolated from the soil by alkali extracts, which contained various specific and non-specific substances. These substances, which are far from being alike, can be destroyed by different groups of microorganisms. They were obtained using fulvic acid on non-polar /activated carbon/ and polar /aluminium oxide/ adsorbents with subsequent dilution of fractions by organic and non-organic dissolvents. The method was described by MURZAKOV /1988/. Four from 13 isolated fractions were attributed to fulvic acids, according to chemical indexes. Their molecular weights range from 1400 to 2200. Their chemical properties are noted in Table 4.

Table 3  
The main diagnostic amino acids and sugars in the hydrolysates of Nocardia cells

Microorganisms	Strains	Diamino- pimelic acid	Lysine	Aspara- ginic acid	Arabino- se	Galac- tose
<i>Øerscovia</i>	7130	-	-	+	-	+
<i>turbata</i>	8	-	-	+	-	+
	67	-	-	+	-	-
<i>Rhodococcus</i>	4274	-	+	+	+	+
<i>sp.</i>	4	-	+	+	+	+
	80	-	+	+	+	+

Table 4  
Elemental and functional composition of fulvic acid fractions

Elemental composition %/	Fractions			
	6	11	12	13
C	45.06	31.10	42.70	25.60
H	6.90	8.97	6.77	4.40
N	5.52	5.83	4.56	1.42
O	42.53	54.10	46.57	68.58
Functional composition: mg/ecv./g				
CaOH	3.26	4.02	5.24	6.34
OH	0.54	0.56	1.30	0.81
OCH <sub>3</sub>	0.464	0.115	0.405	0.47

The fractions of fulvic acids obtained were used to identify the microbial cenosis decomposing them. Some fractions of fulvic acids were added to the mineral medium as the only source of organic substance /0.1% on dry basis/. After 30 minutes' sterilization at 1 atm the prepared media were contaminated with soil suspension. Periodic analyses of the microorganisms growing on the experimental media yielded the information sought about the main destructors of the studied substances and the forms accompanying them, and consuming the decomposition products of humic compounds.

The data obtained allow to conclude that in fulvic acid fractions, with less molecular weight, the main humus destructors are bacteria of the genus *Pseudomonas* and in more complex /higher/ ones representatives of the genus *Rhodococcus*. The accompanying microflora is very various; it includes both common eutotrophs and oligotrophs /Table 5/.

The destruction of humic compounds mainly results from the activity of *Nocardia*-like microorganisms. The properties of these microorganisms are described by ARISTARCHOVA /1989/.

Table 5  
Microorganisms causing the decomposition of fulvic acid fractions and mineralizing the products of their destruction

Fractions	Molecular masses	Main destructors of fulvic fractions	Mineralizators of destruction products
11	1420	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp. oligotrophic bacteria
13	9140	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp. oligotrophic bacteria
12	17120	<i>Nocardia</i> <i>Pseudomonas</i> sp.	<i>Bacillus</i> sp. oligotrophic bacteria
6	22580	<i>Nocardia</i>	<i>Bacillus</i> sp. oligotrophic bacteria

## Conclusions

The experimental data obtained allow to conclude, that new humus formation occurs more actively in the upper layers of the tillage horizon, where the most energetic microbiological processes take place.

The separation of fulvic acids in a number of fractions, which are very distinctive in molecular weight, was carried out by chromatographic method. Microorganisms of the genus *Pseudomonas* form the dominating microflora at the decomposition of less complex compounds. During the destruction of more complex compounds the predominance of bacteria of the genus *Rhodococcus* was established.

## References

- ARISTARCHOVA, U. I., 1989. Nocardia-like microorganisms. Nauka. Moscow.
- MURZAKOV, B. G., 1988. On humic substances' decomposition by soil microflora. *J. Microbiology*. 57. /2/ 292-297.
- VOSTROV, I. S., 1960. Change of microbiological index and soil biochemical activity at its treatment by Maltsev's method. Works of the Inst. of Microbiology, USSR Acad. Sci. 7. 109-120.