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*THE MAIN WAYS OF HUMIC SUBSTANCE DESTRUCTION AND TRANSFORMATION BY LIVING ORGANISMS**

SUMMARY. Humic substances are one of the main accumulators for carbon in the biosphere. They control the global biogeochemical cycle of organic carbon. They also play a crucial role in reduction-oxidation, sorption, complexation, transfer of contaminants and microelements, and plant growth. Humic substances are found in the soil and take part in its formation. Humic compounds are important for many natural processes. Saprotrophic fungi actively contribute to humic compounds degradation. The most important representatives are basidiomycetes and ascomycetes Basidial fungi take part in the process of persistent organics destruction and mineralization, while ascomycetes basically modify and polarize humic substances. The degradation mechanism is connected with a wide spectrum of nonspecific oxidative enzymes, especially the lignolytic exoenzymes-destructors: lignin-peroxidase, manganese-peroxidase, laccase. The article observes the important and scarcely studied issues of humic substance biodegradation by fungi enzymes.

KEY WORDS. Humic substances, biodegradation, fungi, enzymes.

In his works on soil biogeochemistry, V.I. Vernadsky mentioned the importance of living organisms for the changes of chemical structure of soil: "This role is so big that, one way or the other, all the processes in soils are connected with living matter or with its transformation products. In a broader sense, all the processes can be considered biochemical" [1].

The base of soil are humic substances which belong to stochastic organic objects, along with oil and lignin [2]. By their chemical nature, they belong to aromatic oxypolycarbon acid randomized polymers [2, 3]. Due to their specific structure (figure 1) – gydrophobic aromatic carcass and the abundance of functional groups – humic substances demonstrate microligand properties. Their ability to form complexes

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with metal ions [4] defines, as it was mentioned [2, 3, 4], their protective characteristics in biosphere. Humic acids are among the main biospheric reservoirs of carbon, the contents of which are estimated as $1600*10^{15}$r(C). Due to their crucial role in redox, sorption, complexation processes, pollutants and microelements transportation, growth of plants, the studies of humic substances are of highest interest for the modern geochemistry.

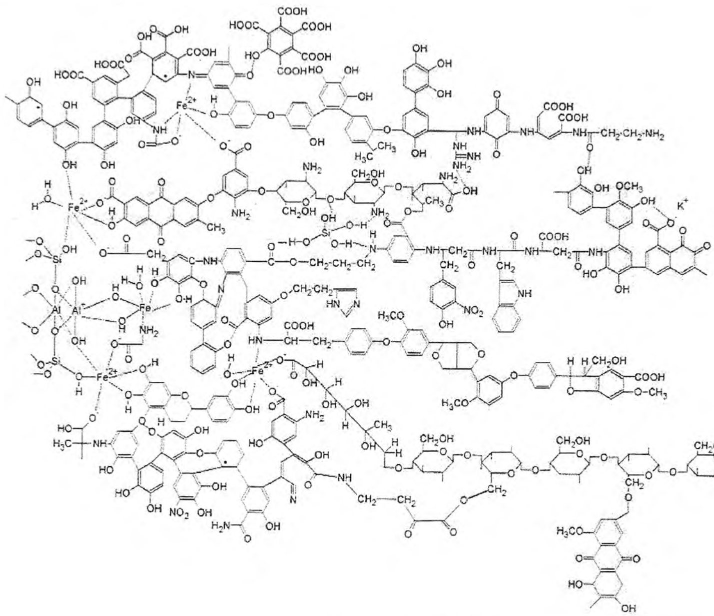

Figure 1. Humic acids' fragment [2].

It is worth mentioning that humic acids participate in soil formation processes, control the global organic carbon biogeochemical cycle. They are very important for many environmental natural processes.

The destruction and transformation of humic substances by living organisms is, according to V.I. Vernadsky, "the result of the perpetual influence of living matter or its transformation products on the chemical processes of soil".

Microorganisms, as the driving force of humic substance formation, transformation, degradation and mineralization, depending on their initial biological properties, have different destructive potential. In his works, Vernadsky underlined the importance of microorganisms for global elements transportation: "It is significant that living matter influences the transportation of the substance the soil needs from the atmosphere". Although bacteria dominate in the environment and participate in humic substances circulation, their capacity to destruct stable macromolecules (over 500 KDa), for instance humic acids or lignin, is limited [5, 6, 7], and so they most effectively destruct low-molecule compounds: humic acid metabolites or fulvic acids. Mushrooms, on the contrary, are very effective destructors of humic acids. Recent studies describing biofilms consortium that were fed with using humic substance as the source of carbon and energy, show that biodestruction takes place as a result of side process or co-metabolism [7].

In natural ecosystems saprotrophic fungi are primary, secondary and tertiary reducents which feed on and process carbon and other bioelements. Such fungi influence the plants continuity and soil stabilization, so they take central place in ecosystem functioning. Nevertheless, there are still some gaps in knowledge on fungi actual biodiversity and function in organic matter destruction. Fungi, taking active part in biodestruction process with ascomycetes and basidiomycetes, are studied for a long time, but nonetheless their relative quantity and role in humic substance circulation is not quite clear. About 8500 described basidiomycete species are lignocellulosic destructors-saprophytes, and near half of them are encountered in soil and higher layer of forest floor [8].

Recent studies [9] connected with the global warming have shown that over the 6-year period of CO_2 quantity increase, the soil carbon has decreased twice, which was caused by the changes in the activity of soil microbal community. The soils containing higher CO_2 also contained more fungi and enzymes that destructed soil carbon. That proves once again the significance of fungi and their role in humic substance natural circulation.

There are numerous results of the studies of humic substance destruction with fungus ferments. It is known that due to their large size, the humic substance macromolecules cannot infiltrate into the cells of microorganisms. That is why the process of their biodegradation occurs under the influence of extracellular enzymes [10]. Many of the basidiomycetes belong to white-rot fungi and also to forest floor destructors. Most of the fundamental studies dedicated to nonspecific oxidative white-rot fungus enzymes, are focused on their role in lignin biodestruction process [11]. Still, there are works examining their important role in organically rich environment, in the process of humic substance formation, transformation and destruction. Besides that, these ferments have been recently studied in association with their ability to destruct different aromatic organic pollutants [3, 10].

Throughout the evolution process the white-rot fungi and the forest-floor destructing fungi have different enzyme systems and that is why they have different capacity of destructing stable organics and different macromolecules influence mechanisms [9, 11].

There are data showing that the groups of lignin-destroying enzymes that are adapted to nonspecific oxidation, such as manganese peroxidase (MnP) and lignin peroxidase (LiP), laccase, participate in destruction processes most effectively. The recent studies with the *Phanerochaete chrysosporium* DNA complete decryption, suggest that some other extracellular enzymes are involved in lignin destruction process. Those ferments (lignolitic system) lead to unstable chemical compounds formation (e.g. phenoxy and carboxy radicals), which later get condensed or polymerized, which actually is the process of humification, or there can be further organic substance degradation with subsequent mineralization [8].

The ways of the fermentative process (destruction or polymerization) depend not only on the ferments or substrates involved, but also on the environmental conditions, such as pH, humidity, oxygen level, conductivity and the presence of other compounds. Due to the unique capacity of nonspecific oxidative ferments to react with different aromatic substrates, the white-rot fungi are the most effective humic compounds destructors [10, 12].

The authors of this article have been the first to show that white-rot fungi are capable of discoloring humic substance solutions and of podzolic soils humic acid macromolecules destructuring. Besides that, the authors have mentioned that aerated cultivation correlates with the degree of degradation [13, 14]. That is confirmed by many other studies of humic acids and lignin destruction.

The work [15] shows that *Trametesversicolor* and *Poria monticola* (brown-rot fungi) are capable of brown coal destructing and dissolving. Another mechanism of brown coal dissolving was discovered, when alkaline metabolites are involved due to acid group ionization or with the involvement of produced chelators that are capable of separating polyvalent ions from coal mass, that leads to the dissolving of the latter.

The authors [16] have used white-rot fungi *Phanerochaete chrysosporium* and humic compounds extracted from Morwell brown coals with different methods using alkaline solutions. Some of the fractions were methylated (using $(CH_3)SO_4$ and CH_2N_2) or demethylated. It was found that *P. Chrysosporium* transforms about 85% dissolved brown coal into low-molecule compounds in 16 incubation days.

Some other white-rot fungi species, including *Nematoloma frowardii b19, Clitocybula dusenii b11, Auricularia sp.* and also RBS 1k and RBS 1b strain basidiomycetes have been studied in a number of works aimed to reveal the ability of those fungi to destructure humic compounds concentrated in coal [17]. Those fungi were capable of destructuring humic acids with nonspecific oxidizing ferments.

Despite the big number of publications on humic substance destruction, any reliable description of quantitative and qualitative specifics of the process could not be found. On the one hand, the process of destruction under the influence of UV radiation and peroxy-compounds is well studied, due to relative simplicity of experiments and clear results. On the other hand, many works are done on synthetic humic substance destruction with fungus ferments, and that only approximately reflects the processes that occur naturally. For the modern geochemistry, the most important areas of studies are: 1) the kinetics of humic substance biochemical destruction under the influence of ferments, as it is close to natural processes; and 2) destruction changes due to anthropogenic influence: acidification, increased content of heavy metals, etc.

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